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From willow to aspirin: what history teaches us about the future

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Abstract

The story of aspirin may have started about 5000 years ago with a description of the therapeutic use of willow in case of pain and fever. Throughout history willow bark and meadowsweet were used in different forms in phytotherapy. Only after the chemical isolation of salicylic acid from these plants in the 19th century could a phytochemical approach be applied. At the end of the 19th century, acetylsalicylic acid was synthesized from salicylic acid. Soon the substance became known worldwide as an analgesic, antipyretic and anti-inflammatory medicine. Despite its success, it took more than 70 years to discover the mechanism of action of acetylsalicylic acid. Discovering this mechanism led to new therapeutic developments, more particularly, the use as an antiplatelet drug that could protect patients with cardiovascular risks against myocardial infarction and cerebral thrombosis. In the future aspirin may play an important role in the prevention of cancer and chronic inflammatory diseases.

This narrative review demonstrates how careful observations, ethnopharmacological evaluation of the use of medicinal plants (and more particularly willow species), phytochemical investigation and pharmacological insight are a guarantee for scientific development for the profit of patients.

History

The use of willow in ancient Egypt

Edwin Smith was an American who moved from Connecticut to Egypt and did quite good business in the 19th century buying and selling all kinds of souvenirs to the tourists, who were starting to come to Egypt to admire the intangible heritage of the Pharaohs' kingdom. He had studied Egyptology and could even read the written language of pictograms and hieroglyphs. By chance he bought from locals, who continuously robbed the graves in the pyramids, two tattered papyrus scrolls for £12. They were a bargain, as he discovered by studying their content. The papyrus scrolls described the symptoms of human pathological conditions and about 160 herbal

remedies that could be used to treat them. Smith identified willow as one of the most important medicinal plants. The scrolls dated back to about 1534 BC but the practice of using willow probably dates from much earlier. References within the text suggest that they were inspired by manuscripts written at the time of the Old Kingdom, around 3000 BC. The merit of the papyrus scrolls is that they combine particular diseases with herbal remedies. The scrolls were subsequently known through history as the Ebers Papyrus, named after the German professor who later acquired them from Smith (Jeffreys, 2005).

Because of their knowledge, Egyptian physicians were in demand and much imitated during the Greek and Roman periods. Hippocrates practiced on the Greek island of Kos in the 5th century BC. He wrote the *Hippocratic Corpus* (Hippocrates, *sine dato*), a series of medical texts listing diseases, diagnoses and treatments. The Roman physician Celsus used extracts of willow leaves around 30 AD to relieve what he considered as the classical signs of inflammation, *rubor*, *calor*, *dolor* and *tumor* (respectively, redness, warmth, pain and swelling). Dioscorides, a Greek physician who served in the armies of Emperor Nero, described the therapeutic potential of willow in his *De Materia Medica* (Dioscorides, *sine dato*), linking willow to the inhibition of the symptoms of inflammation. This work was translated by the Arabs. During the 10 centuries that came after the disintegration of the Western Roman Empire, the knowledge that served so many patients entered the darkness of ignorance. Fortunately, the practice of using willow continued in the Arab world and it might have partially come back into fashion after the migration of medical doctors to the western world following the fall of Byzantium in the 15th century.

Reintroduction of the medical use of willow in Europe

Observation was the main source of knowledge after the Medieval Ages, and it became possible to spread knowledge more easily after the invention of book printing in the second half of the 15th century. Many interesting books about herbals and their properties were published. One of them was the '*Cruydt-boeck*' (Herbal Medicines Book) by Rembert Dodoens (1517 - 1585), a medical doctor and professor of botany at the University of Leiden, Netherlands. Dodoens described how the leaves of the willow should be used (free translation from Dutch):

... The fresh green leaves of willow should be used as twigs that must be spread in the room and around the bed of the sick patient who suffers from high fever. These twigs have a modest cooling effect. They will refresh the air in the room and, by this, strongly relieve the patient who is tortured by an unbearable feeling of hotness and inflammation and give him support ...

(Dodoens, 1608).

It may seem strange to use twigs to refresh the air but, knowing now that willows contain salicylaldehyde and that aldehydes are relatively volatile, it is not so surprising that the odor of the willow twigs could have influenced the feeling of the patient.

Dodoens (1608) describes another practice that is closer to the actual use:

… The bark of these willows has a similar potency as the leaves. When it is burned to ash, it can protect against warts and makes disappear corns, when the ash is put on them …

We know that willow bark is a natural source of salicylic acid and when the bark is not completely reduced to inorganic ash, it can still contain organic material like salicylic acid. The practice of using this substance on warts and corns is still valid, as it facilitates the removal of keratinic eruptions caused by papilloma viruses in the case of warts and softens the hardened skin in the case of corns (Cockayne *et al*., 2011).

In 1763 the Royal Society of London reported on the content of a letter written by the Reverend Edward Stone, living in Chipping Norton, in which he wanted to let experienced scientists know about the properties of willow:

… There is a bark of an English tree, which I have found by experience to be a powerful astringent, and very efficacious in curing agues and intermitting disorders. About six years ago, I accidentally tasted it, and was surprised at its extraordinary bitterness, which immediately raised in me a suspicion of it having the properties of the Peruvian bark. As this tree delights in a moist or wet soil, where agues chiefly abound, the general maxim that many natural maladies carry their cures along with them, or that their remedies lie not far from their causes, was so very apposite to this particular case, that I could not help applying it … (Stone, 1763).

Stone pointed to the incidence of the tropical infection, malaria, in Western Europe, which until a century ago occurred during the summer in swampy regions. He successfully applied willow bark to combat fever as a symptom of the infection.

Phytochemical insight, a successful synthesis and a green light

In the 19th century chemistry took the lead in science. First salicin was obtained as bitter-tasting yellow crystals. It is a secondary metabolite in willow consisting of a compound of salicylic acid and glucose. The binding to glucose makes salicylic acid more water-soluble so that it can be transported in the tree. It is further converted to salicylic acid (Fig. 1). Salicylic acid was isolated from another plant, called meadowsweet (*Spiraea ulmaria* L. or *Filipendula ulmaria* (L.) Maxim.) by Karl Jacob Lowig of Berlin. The name of the plant at the time, *Spiraea ulmaria*,

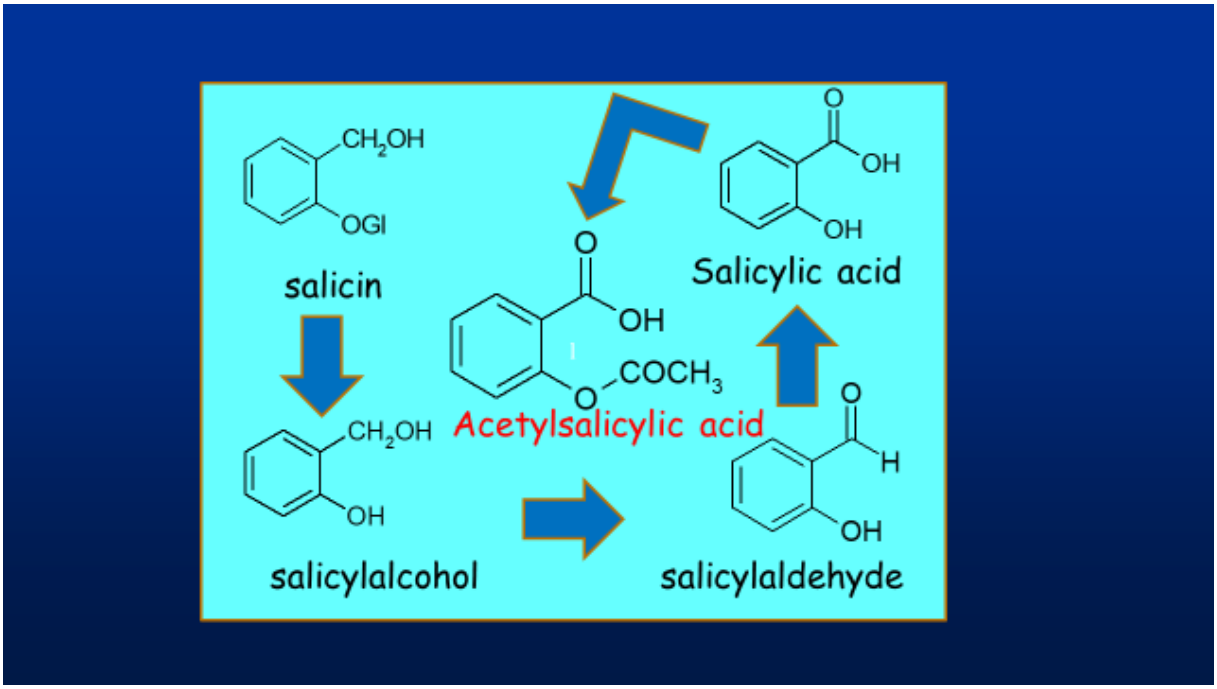

Figure 1. Chemical interactions of salicylic acid derivatives present in willow bark and meadowsweet. Salicin is a soluble form because of its linking to glucose (Gl). Salicin is converted to salicylalcohol by hydrolysis. Salicylalcohol is subsequently oxidized to salicylaldehyde, which reacts further to salicylic acid. All these reactions take place in the salicin-containing plants. The last step to acetylsalicylic acid consists of a reaction of salicylic acid with acetic acid anhydride, which has to be performed in the laboratory or reactor.

contributed to the naming of aspirin. Once salicylic acid was available as a chemical substance, it was therapeutically used as a substitute for willow and meadowsweet as its sodium derivative (sodium salicylate). Unfortunately, the substance was so corrosive for the stomach that it could not make its way to the medicines market. As the hydroxyl group (OH) was suspected of causing the dreadful gastric irritation, a French scientist called Charles Gerhardt, Professor of Chemistry at Montpellier University, tried to replace the hydrogen atom with an acetyl group in 1853. Unfortunately, laboratory techniques in the 19th century were not sufficiently sophisticated to obtain enough yield or to separate the desired product, acetyl salicylic acid, from the complex mixture (Jeffreys, 2005).

It was Felix Hoffmann who, 44 years later, wrote in his laboratory journal:

... When salicylic acid (100,0 parts) is heated with acetic anhydride (150,0 parts) for 3 hours under reflux, the salicylic acid is quantitatively acetylated. After distilling off the acetic acid one obtains the above in the form of needles, which, when crystallized from benzene, melt at 136 degrees. In contrast with the literature reports, my acetyl product no longer gives a reaction with ferric chloride, which readily distinguished it from salicylic acid. By its physical properties, e.g.

its sour taste without being corrosive, the acetylsalicylic acid differs favorably from salicylic acid, and is now being tested in this respect for its usefulness … (Hoffmann, 1897).

Felix Hoffmann was working in the pharmaceutical chemistry laboratory of the drug and dye company, Bayer, where he was strongly supported by his head of department, Arthur Eichengrün. The recipe jotted down by Hoffmann gave a high enough yield for the process to be industrialized, but before the substance went into industrial production, it had to be tested for its pharmacological activities in the laboratory led by Heinrich Dreser. Eichengrün thought the substance was ready for clinical testing but Dreser thought it might enfeeble the heart and gave negative advice. It came to a clash between the two heads of department. According to Dreser, it would be better to concentrate on another substance, more particularly, diacetylated morphine or diamorphine, which later became known as heroin. Dreser was convinced that this substance had a much better commercial potential. Eichengrün decided to take the initiative into his hands by contacting Felix Goldman, Bayer's representative in Berlin, who facilitated contacts with clinicians who performed discreet clinical trials. It was seen that acetylsalicylic acid did not have the unpleasant effects of salicylic acid whilst retaining the beneficial effects. Soon it was considered as a promising general-purpose analgesic. Once the clinical reports started to circulate among the laboratory staff of Heinrich Dreser, the latter was intelligent enough to accept the evidence. The way was cleared for a new drug. As already mentioned, the brand name was inspired by *Spiraea*, the Latin genus name of meadowsweet. The 'a' was added to refer to the acetylation process. The wonder drug was baptized and started its world tour in 1899. Although he had been a strong opponent of the drug, Dreser wrote a pre-launch paper glorifying the therapeutic benefits of the new medicine. This paper is acknowledged as a scientific classic, a wonderful exposition of the medicine's chemical composition, test history and benefits (Dreser, 1899). It brought the acetylsalicylic acid to the attention of doctors and pharmacists and contributed significantly to its worldwide success. Unfortunately, Dreser completely omitted the contribution of Eichengrün and Hoffmann in this important paper. Furthermore, Dreser negotiated a special deal that ensured he was paid royalties on all medicines tested in his laboratory. He became rich, whereas his colleagues got nothing (Jeffreys, 2005).

Mechanism of action and new perspectives

In 1956 the Monsanto Chemical Company produced its 100 millionth pound of aspirin. At the celebration of this event, Dr. Carrol A.Hochwalt, Monsanto's head of research and development, gave the keynote speech. He concluded:

… There is still much to be learned of the compound's mechanism of action. How does it do what it does for the rheumatic and arthritis sufferers? Why does it reduce abnormal body temperatures while having no effect on normal temperatures? Just what is the means by sure to come in time and with them may come still more important applications for this cheapest, safest and most perennially durable of the genuine wonder drugs … (Jeffreys, 2005)

The world had still to wait for 15 years, before the mechanism of action of acetylsalicylic acid was discovered by a constructive collaboration of British scientists. An interesting aspect of the story was the simple technique by which the discovery was made. John Vane was the head of the laboratory of pharmacology at the Royal College of Surgeons in London. One of his notable achievements was the development of a new way to conduct bioassays – at that time, the only method to detect the activity of unstable biological agents formed during the stimulation of organs was by using isolated tissues of laboratory animals. Vane and his collaborators conducted experiments with guinea pig lung tissue because this animal has an immunological system that is similar to that of humans. Guinea pigs could be made allergic to the egg protein ovalbumin. After two to three weeks of injecting small amounts of emulsified ovalbumin into their feet the guinea pigs become allergic to it. When taking the lungs out of the animal, these organs can be stimulated with ovalbumin to give an allergic reaction called anaphylaxis. As a consequence, biological substances are released, some of them very unstable with an activity half-life of barely 30 seconds. During the stimulation, a solution containing salts and glucose at physiological concentrations was dripped onto the anaphylactic organ and could then carry the released agents over other isolated tissues, including a piece of rabbit aorta. Chemicals could be added to the torrent, each of which was known to neutralize one or other of the complex agents released during the anaphylactic reaction (Fig. 2). Vane and his collaborator Priscilla Piper noticed that one component seemed to escape from the neutralization as it kept contracting the rabbit aorta. They called the agent rabbit aorta contracting substance or RCS. Priscilla Piper suggested trying aspirin in the experimental model and to the astonishment of Vane and Piper, aspirin neutralized the mysterious RCS.

This observation had to be further explored. The Swedish scientist Sune Bergstrom had done valuable work on a class of substances called prostaglandins between 1950 and 1960. He had shown that prostaglandins were generated from arachidonic acid, a substance that occurs in living cells as an essential component of cell membranes. When cells are irritated or injured, it is released and converted to prostaglandins. It emerged that prostaglandins were essential in the

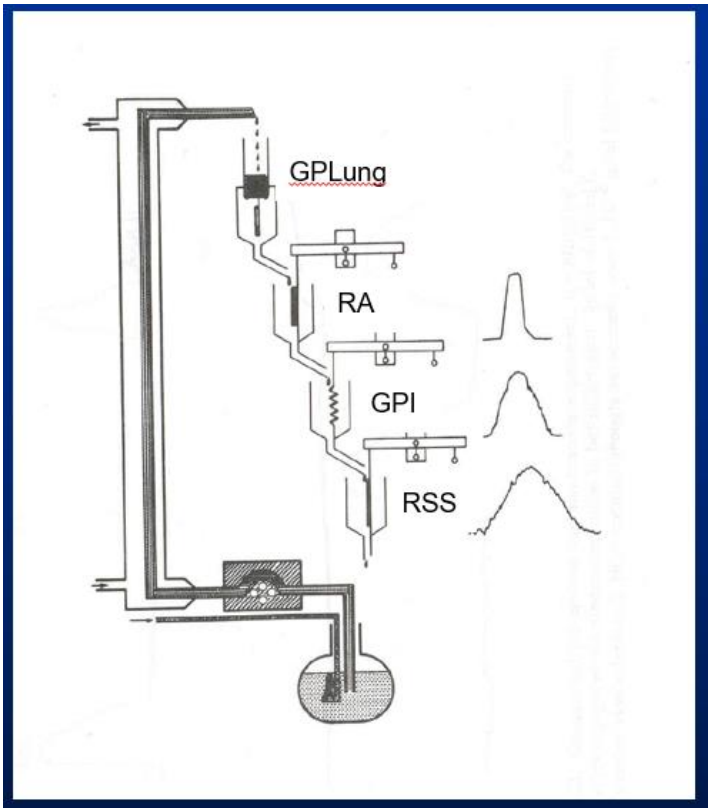

Figure 2. John Vane and his collaborators discovered the mechanism of action of aspirin by using a cascade technique. In the cascade a series of isolated organs and tissues is continuously wetted with a physiological oxygenated solution at 37°C. When the isolated lung of anaphylactic guinea pig (GPLung) is triggered with ovalbumin, reactive agents are released and then contact the organs underneath. In the example non anaphylactic tissues like rabbit aorta (RA), guinea pig ileum (GPI) and rat stomach strip (RSS) are used as detectors.

regulation of many important functions in the body, such as the elasticity of blood vessels, uterine contractions and all kinds of inflammatory reactions. It was suspected that some prostaglandins produced effects similar to the ones seen in Vane's various bioassays. Two years after the discovery of RCS, Vane got a flash of insight. What if RCS was a still-undiscovered prostaglandin? Furthermore, what if aspirin inhibited the creation of prostaglandins in general? In that case, aspirin was interfering with inflammation, one of the most important processes in the body! Vane sent a letter developing these thoughts to the editor of *Nature*, the world-famous scientific journal. Vane's genuine eureka moment changed the face of aspirin. It was an explanation of what Celsus and Dioscorides had been observing nearly 2000 years earlier, linking the activity of willow to the inhibition of the symptoms of inflammation (Vane, 1971).

In the years that followed much of the mystery about the activity of aspirin would be unraveled. Vane and his team took the lead in this research, although also other research centers competed in the race. They established that aspirin blocked the activity of cyclooxygenase (COX), the enzyme that generates prostaglandins from arachidonic acid. In 1982, John Vane got the Nobel Prize for Medicine together with Sune Bergstrom and Bengt Samuelsson, also from the Swedish Karolinska Institutet in Stockholm, who investigated another pathway of arachidonic acid metabolism (Samuelsson, 1987).

In doses between 300 and 600 mg, aspirin acts mainly on pain, fever and inflammation. However, intensive research enlarged the therapeutic applications. Aspirin is a powerful

medicine, but patients have to accept the undesirable effects such as gastrointestinal irritation that can lead to serious complications like gastric bleeding and perforations. Despite the discovery of its mechanism of action, aspirin was gradually replaced by substances that did not show the gastric complications of aspirin. Acetaminophen or paracetamol was one of them. On the other hand, the discovery of thromboxane A_2 as an important pro-aggregating agent in blood platelets opened new perspectives. Blood platelet aggregation in the coronary arteries was considered as a triggering mechanism for the formation of clots that could hamper circulation in blood vessels. Subsequent damage of the heart muscle by lack of oxygen would then lead to life-threatening rhythm disturbances. It was proposed to give aspirin to patients who suffered from such a myocardial infarction in order to prevent a second cardiovascular incident. Despite the positive results of the initial clinical trials, there was some reluctance, as the therapeutic effect seemed modest and many patients had to give up taking aspirin because of gastrointestinal complaints. Nevertheless, some of the trials were published and contributed to the scientific discussion (Peto *et al.*, 1988). Finally, aspirin got a therapeutic cardiovascular indication after the daily dose was lowered to 80 or 100 mg and specific marketing authorizations were granted. In these medicines, acetylsalicylic acid is in a gastric-resistant tablet that only disintegrates in the intestine instead of the stomach, thus avoiding direct damage to the gastric wall. After its partial waning from the analgesic and antipyretic market, aspirin has been re-emerging with new therapeutic goals. From the nineties onwards, aspirin found its second breath. It is now widely recommended as a basic anti-aggregating drug in cardiovascular patients (ATT Collaboration *et al.*, 2009).

Because of the role of inflammation in the development of diseases, aspirin may help to protect against pathological conditions other than cardiovascular ones. The worldwide use of low-dose aspirin may help to get insight into this matter by epidemiological observation and analysis. As a consequence, new therapeutic uses may be developed.

Conclusion

Willow and inflammation were therapeutically linked to each other some 2000 years ago. History teaches us that: (1) natural resources, in this case willow, are important sources of therapeutic compounds; (2) observation is one of the most crucial issues in the development of practice; (3) chemistry contributes to the development of therapeutically active substances from plants; (4) linking phenomena is important to make discoveries; (5) simple techniques can be useful in gaining insight; (6) patience is a weapon against discouragement in scientific research.

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